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(54) GLUCOSE ENDOCYTOSIS INHIBITOR AND GLUCOSE TRANSPORTER 4 TRANSLOCATION INHIBITOR IN FAT CELL, GLUCOSE ENDOCYTOSIS ACTIVATOR IN MUSCLE CELL, AND FAT-REDUCED FOOD AND DRINK

(57)Abstract:
PROBLEM TO BE SOLVED: To provide new applications of an extract of tea and components thereof, based on results of studies on effects of the tea and the components on glucose endocytosis activity, functions of glucose transporter 4 (GLUT4), or the like.
SOLUTION: A glucose endocytosis inhibitor in fat cells containing catechin gallate as an active ingredient and a GLUT4 translocation inhibitor in the fat cells containing any one of the catechin gallate, catechin together with a gallate ester, and the tea extract as an

active ingredient are provided in the specification, respectively. The inhibitors each reduce an amount of fat cells, so that obesity and various diseases caused by the obesity are treated and prevented by the inhibitors. On the other hand, the inhibitors activate the endocytosis of the glucose in the muscle cell, so that the excess glucose is endocytosed into the muscle cell and consumed. Therefore, the inhibitors never increase sugar concentration in the blood, nor give lassitude caused by obesity control.

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CLAIM + DETAILED DESCRIPTION

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[Claim(s)]
[Claim 1] Glucose taking-in inhibitor in the adipose cells which make catechin gallate an active ingredient.
[Claim 2] Insulin stimulus response nature glucose taking-in inhibitor which makes catechin gallate an active ingredient.
[Claim 3] The GLUT4 transformer location depressant in the adipose cells which make an active ingredient catechin gallate, catechin equipped with gallate ester, or a tea extract.
[Claim 4] The glucose taking-in activating agent in the muscle cells which make an active ingredient catechin gallate, catechin equipped with gallate ester, or a tea extract.
[Claim 5] Fat mitigation food and drink which adds the catechin gallate which isolated.

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[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the tea extract obtained by extracting tea (Camellia sinensis) and the new use of the component, especially the new use in taking in of glucose.
[0002]
[The background of invention, and a Prior art] "Overweight" is one of the most serious troubles that a man of today holds as eating habits become rich. Causing various illnesses [, such as a disease ], such as about [ that overweight is not desirable in cosmetics ], diabetes, arteriosclerosis, high bird acyl glycerol ****, high cholesterol ****, and thrombosis, is known.
[0003] Although overweight is produced by the increase in specialization and hypertrophy of adipose cells, or the number of adipose cells itself, in any case, "taking in of glucose" is involving deeply.
[0004] By the way, since glucose is a polar substance, a transportation carrier (gulucose transporter:GLUT) needs glucose into each cell out of blood to be taken in. The cloning of nine kinds of GLUT(s) (GLUT 1-9) is carried out now, and GLUT1 and GLUT4 are discovered to the adipose cells which participate in sugar and lipid metabolism in the living body greatly in it. Especially GLUT4 have played main roles in the taking-in

activity of glucose on the film of adipose cells also in it.
[0005] GLUT4 are called insulin reception type GLUT, they usually exist in the intracellular small granule vesicle in adipose cells and muscle cells, and if a stimulus of an insulin is received, they will change into the state where it shifts on a cell membrane (transformer location), and glucose can be taken in. An insulin combines the transformer location of GLUT4 with a receptor. It becomes the start of communication of information that beta subunit of a receptor carries out self-phosphorylation. Shift is completed by exoSAITOSHISU from an intracellular endoplasmic reticulum to a cell membrane through the course of phosphorylation of an insulin receptor ground substance (IRS), activation of phosphaCHIJIRUINOSHITORU 3 KINAZE, and activation of Akt/Protein KinaseB after that. Moreover, GLUT4 succeed in the work to which much glycogen is moved by the inside of muscle cells, and it is reported that an athlete has more GLUT4 than ordinary persons ("newest knowledge of carbohydrate loading" Keio University sports medicine research center bulletin 1996).
[0006] From the above point, as a result of a tea extract and its component inquiring about the influence which it has on the taking-in activity of glucose, the function of GLUT4, etc., this invention person can get various discovery and used to come to hit on an idea of this invention based on the result.
[0007] Incidentally about the function of a tea extract, [ JP,H06-80580,A ] conventionally The plasma cholesterol fall agent which makes an active ingredient the polysaccharide (RIBOSU, arabinose, and glucose) contained in tea leaves is indicated. [ the fat solution catalyst containing the vegetable extract which JP,H10-158181,A extracted from tea ] It indicates promoting reduction of the adipose tissue of the whole body or a part, and demonstrating an effect to an improvement of inclination to grow fat, and control and prevention of overweight. The glucose absorption inhibitor with which JP,H11-302168,A contains epicatechin gallate in a tea extract as an active ingredient controls the glucose absorption by an intestinal tract, and is indicating the purport effective in the medical treatment of overweight, diabetes, etc.
[0008]
[Means for solving problem] This invention proposes glucose taking-in inhibitor and insulin stimulus response nature glucose taking-in inhibitor in the adipose cells which make an active ingredient catechin gallate, catechin equipped with gallate ester, or a tea extract.
[0009] If the active ingredient of this invention is taken in, taking in of glucose in adipose cells and glucose taking in increasing [ with especially an insulin stimulus ] can be controlled, and the amount of adipose cells can be reduced. By this, the medical treatment and prevention of various illnesses [, such as a disease, ], such as diabetes accompanying an overfat state, i.e., overweight, and overweight, arteriosclerosis, high bird acyl glycerol ****, high cholesterol ****, and thrombosis, can be aimed at. furthermore, the active ingredient of this invention not only controls glucose taking in in adipose cells, but [ an active ingredient ] Glucose taking in in muscle cells is activated conversely, and since superfluous glucose can be made to be able to take into muscle cells and can be made to consume, improvement in activity can also be aimed at [ that blood sugar concentration is not raised and there is no feeling of worthlessness accompanying overweight prevention etc., and ] conversely.
[0010] Although various mechanisms, such as control of the transformer location of the

glucose transportation carrier which exists in adipose cells, or combination of insulin RESEPUTAHE, can be considered as a mechanism which checks glucose taking-in activity in adipose cells In this research, it solved that the active ingredient of this invention controlled the transformer location of GLUT4 specifically. Then, this invention proposes the GLUT4 transformer location depressant in the adipose cells which make an active ingredient catechin gallate, catechin equipped with gallate ester, or a tea extract.
[0011] Moreover, this invention offers the glucose taking-in activating agent in the muscle cells which make an active ingredient catechin gallate, catechin equipped with gallate ester, or a tea extract. If this active ingredient is taken in, the GLUT4 transformer location in muscle cells can be activated, the taking-in activity of glucose in muscle cells can be increased, as a result many energy sources can be made to be able to take into muscle cells, and the amount of muscle cells can be increased. Therefore, if it reinforces and increases and activation of the muscular system, flesh fatigue mitigation, improvement in an athletic ability, and the muscular system pull, it can use to constitution reconstruction etc. effectively. Therefore, it can provide also as catechin gallate, catechin equipped with gallate ester, the GLUT4 transformer location activating agent in the muscle cells which make either of the tea extracts an active ingredient, or a muscular activating agent.
[0012] The catechin equipped with catechin gallate and gallate ester as an active ingredient in above-mentioned this invention, Although what is necessary is just either of the tea extracts, also especially the catechin preferably equipped with gallate ester, and in it, catechin gallate can expect a high effect also in invention [ which ], and can be said to be desirable.
[0013] Since it is checked that an effect can be acquired in any by this research before and after insulin secretion even if it takes in the active ingredient of this invention, even if it takes in the active ingredient of this invention to any after ingestion, simultaneous, or ingestion, it can acquire an effect before ingestion. And the active ingredient of this invention is drunk habitually daily for many years, it can be taken in in comfort that there is no prolonged unreasonableness since it is an ingredient originating in the tea which can take in whom in comfort, and is effective for the fundamental medical treatment of a chronic condition and the illness and prevention, and also especially improvement of physical condition.
[0014] In addition, catechin of this invention is the mind of (-)-catechin, and when the display of (-) is omitted, it means (-)-catechin. For example, catechin gallate is the mind of (-)-catechin gallate.
[0015]
[Mode for carrying out the invention] "Insulin stimulus response nature glucose taking-in inhibitor" of this invention, "glucose taking-in inhibitor in adipose cells", "The GLUT4 transformer location depressant in adipose cells", "the glucose taking-in activating agent in muscle cells", Each of "GLUT4 transformer location activating agents in muscle cells" and "muscular activating agents" can be manufactured by blending suitably a "tea extract", "catechin equipped with gallate ester", or "catechin gallate" by concentration.
[0016] a "tea extract" -- tea (the tea leaves of Camellia sinensis --) a tea bud, a tea plant, etc. -- containing -- it is what is extracted and obtained -- fermentation tea, such as a tea green leaf, tea, and PUARU tea, -- A mixture can be used although obtained by having extracted either of the non-fermented tea, such as half-fermentation tea, such as oolong

tea and ******, green tea, and boiling-in-an-iron-kettle green tea, roasted tea, (independent), the thing obtained by extracting two or more kinds of these mixtures, or each. However, it is desirable to use not a narcissus but the iron Kannon and a color type also in the inside of the inside of green tea or coarse tea, not highest-quality green tea but green tea, and tea or Dimbula, and not Uva but NUARA and oolong tea, considering the effect of this invention, for example, the point of insulin stimulus response nature glucose taking-in inhibitory action.

[0017] moreover, a "tea extract" -- tea -- water, warm water, or **** -- desirable -- warm temperature water (40 degrees C - 100 degrees C) -- By refining means, such as an extract especially extracted and obtained in 90-100-degree C ****, and distribution extraction which uses this extract for filtration or ethyl acetate of resin adsorption, ultra filtration, reverse osmosis filtration, etc., etc. still more preferably, catechin, The tea extraction extract which condenses or dried catechin equipped with inside or gallate ester, the tea extracts refined and obtained also in it in the direction which raises the content of (-)-catechin gallate, or these tea extracts can be mentioned. The green tea extract (Ito En brand name: Thea Fran 30A) which carried out heat water extraction processing of the green tea, was made to dry this extract and made catechin concentration about 30% as a desirable example of a green tea extract, Heat water extraction processing of the green tea can be carried out, in order to eliminate ingredients other than catechin, this extract is processed with a column method and can be dried, and the green tea extract (Ito En brand name: Thea Fran 90S) which made tea polyphenol concentration about 85 to 95% can be illustrated.

[0018] [ catechin / on the other hand, / "catechin equipped with gallate ester" ] (-) - epicatechin gallate, (-)-epigallocatechin gallate, (-) - catechin gallate or (-)-GAROKATEKIN gallate Or what includes two or more kinds of two or more kinds of ones of these polymers or copolymers of these and mixtures of these, and contains (-)-catechin gallate 25% or more also in it, and "(-)-catechin gallate" which isolated further are desirable. "Catechin equipped with gallate ester" exists in [ other than tea ] many plants, such as Kola, large yellow, an apple, a peach, a pear, a cocoa bean, and ******, and it can also be obtained by the ability to extract these. If retort sterilization of above-mentioned catechin or those above-mentioned mixtures is carried out, it is known that the polymer and copolymer of these catechin will arise.

[0019] Although the above "tea extract", "catechin equipped with gallate ester", and "(-)-catechin gallate" can be blended as a respectively independent active ingredient Already or in the future Insulin stimulus response nature glucose taking-in inhibitory action, The glucose taking-in inhibitory action in adipose cells, the GLUT4 transformer location depressant action in adipose cells, It is also effective to mix with the material the glucose taking-in activation operation in muscle cells, the GLUT4 transformer location activation operation in muscle cells, or the muscular activation operation was accepted to be, and to blend these as an active ingredient. In addition, a "tea extract", "catechin equipped with gallate ester" when blending as an independent active ingredient, It can dissolve in refining water or a physiological saline, and "(-)-catechin gallate" can be offered as medicines (for example, an internal use agent, an intraperitoneal injection agent, an intracerebral administration agent, etc.) etc., respectively.

[0020] "Insulin stimulus response nature glucose taking-in inhibitor" of this invention, "glucose taking-in inhibitor in adipose cells", "The GLUT4 transformer location

depressant in adipose cells", "the glucose taking-in activating agent in muscle cells", "the GLUT4 transformer location activating agent in muscle cells", As for each "muscular activating agent", it is desirable to consider it as the combination and the drug design which could use it as an internal use agent or parenteral medication agents (an intramuscular injection, an intravenous injection, hypodermic administration, rectum medication, endermic medication, pernasal medication, etc.), and were suitable for each medication. Speaking of a drug design, for example as an object for internal use agents, it can prepare in the form of liquid medicine, a tablet, powder medicine, granulation, a sugar-coated pill, a capsule, soil suspension, an emulsion, a pill, etc., and can prepare as an object for parenteral medication agents in forms, such as an injectable solution, an ampul agent, a rectum medication agent, a ******** agent, and a ******** agent. Speaking of combination (tablet), it can manufacture by a usual state method using the diluent base and extender which are usually used, a binding material, a humid-ized agent, disintegrator, a surface-active agent, lubricant, a dispersing agent, a buffer, a preservative, a solubilizing agent, an antiseptic, correctives, a soothing agent, a stabilizer, etc. Moreover, for example, milk sugar, fructose, grape sugar, starch, gelatin, magnesium carbonate, A synthetic magnesium silicate, talc, stearic acid magnesium, methyl cellulose, It is also possible to blend avirulent additive agents, such as carboxymethyl cellulose or its salt, gum arabic, polyethylene glycols, syrup, vaseline, glycerin, ethanol, propylene glycol, citrate, sodium chloride, sodium sulfite, and sodium phosphate.
[0021] Moreover, "insulin stimulus response nature glucose taking-in inhibitor" of this invention, "glucose taking-in inhibitor in adipose cells", "The GLUT4 transformer location depressant in adipose cells", "the glucose taking-in activating agent in muscle cells", "the GLUT4 transformer location activating agent in muscle cells", Each "muscular activating agent" can also be offered as medicines to an animal, feed, etc. other than an unregulated drug besides medical supplies, health food, a health drink, a food for specified health use and functional foods equipped with the medicinal value, and other humans. For example, it can be made much more easy to take in by preparing as an unregulated drug and making this into the form of drink forms, such as a bottle drink drink, or a tablet, a capsule, granulation, etc. As health food, a health drink, a food for specified health use, and functional foods equipped with the medicinal value The active ingredient of this invention For example, a carbonic acid, a diluent base (granulation agent ****), a dilution agent, Further Or various protein, such as a sweetener, a flavor, flour, starch, sugar, and oil and fat, [ mix with a kind chosen from eating-and-drinking article material groups, such as non-fibrous-carbohydrates materials, and vitamin, a mineral, or two sorts or more, or ] An eating-and-drinking article well-known now, for example, a sport drink, a fruits drink, a milk beverage, a tea drink, It can add and manufacture to vegetable juice, a lactic beverage, an alcoholic beverage, jelly, a jelly drink, a carbonated drink, chewing gum, chocolate, Kandy, a biscuit, a snack, a bread, dairy products, fish meat boiled fish paste, a meat product, ****, a dried food, a supplement, etc. The food and drink which adds "the catechin gallate which isolated" for the food-and-drink material having contained many sugars can be especially offered as the outstanding fat mitigation food and drink (if it puts in another way diet food and drink), muscular activation food and drink, etc.
[0022] [ content ] although the content of the active ingredient in this invention changes also with directions for use It is desirable to make it catechin dry weight conversion and

to blend 0.01 to 0.5weight % especially 0.001 to 1weight %, if it is medical supplies, and if it is an eating-and-drinking article, it is desirable to make it catechin dry weight conversion and to blend 0.01 to 0.5weight % especially 0.001 to 1weight %. When preparing as fat mitigation food and drink or muscular activation food and drink, it is desirable to blend the catechin gallate which isolated 0.001 to 1weight % into an eating-and-drinking article, and to prepare catechin gallate concentration by 5 times - 500 times the tea usually drunk. In addition, as intake, about 100-1500mg will be preferably desirable 10-5000mg at catechin dry weight conversion on the first.

[0023] In the <examination 1> exam, in order to investigate change of the sugar metabolism at the time of making Latt do free ingestion of the green tea extract, and lipid metabolism, change of taking in of glucose in a constituent of blood and adipose tissue, and the muscular system was investigated.

[0024] (Latt's breeding) Wistar system male Latt immediately after ablactation (3 weeks old) was used for the examination. these [ five ] were divided into each two groups, and one group was made to carry out free ingestion of the ion exchange water for a green tea extract (Ito En: -- " - be -- tea") for three weeks as control at another side, respectively Weight, an amount of food ingested, and ****** were measured every day. After anesthetizing Latt who made it abstain from food for 4 hours three weeks afterward and collecting blood from the heart, it made an incision in the abdomen, adipose tissue and a muscle of thigh were extracted, and it used for the experiment.

[0025] (Measurement of a plasma ingredient) Centrifugal separation separated the blood which collected blood into a blood cell and plasma. The blood sugar level, the amount of bird acyl glycerol, the amount of free fatty acid, the amount of total cholesterol, and the amount of HDL cholesterol were measured using the Wako Pure Chem measurement kit using the obtained plasma.

[0026] After extracting adipose tissue and the thigh muscular system from Latt, (Taking in of 3-O-Methyl-D-glucose (it is called the following "3-OMG".) in adipose tissue and the muscular system) The fragment was carried out and about 100mg organization small piece was incubated for 80 minutes in Krebs-Ringer-Hepes buffer (KRH) containing glucose of 5.5mM. Furthermore, it transposed to KRH which does not contain glucose, and incubated for 15 minutes, and 3-OMG (6.5mM, 0.5microcurie) which carried out the label by 3H was made to take in for 30 seconds. Then, taking in was stopped with the KRH solution of 0.3mM FURORECHIN, and it was made to dissolve completely with a NCSII solubilizing agent after several times washing with this solution. The amount of 3-OMG(s) taken into the in-house in liquid scintillation counters was measured after the dissolution.

[0027] In order to investigate ****** influence on a cell level to the glucose transport mechanism of a <examination 2> tea ingredient, The 3T3-L one-share cell which is a precursor adipose cell of mouse origin was made to specialize in adipose cells, and it investigated about taking in into the cell of glucose when adding a "tea extract" or "catechin" to this, and the transformer location of GLUT4.

[0028] (Manufacture of a tea extract) As a "tea extract", the extract of green tea (green tea (from Motoyama), highest-quality green tea (from Asahina), and coarse tea (from Shizuoka)), tea (NUARA Uva Dimbula), and oolong tea (the iron Kannon, a color type, and narcissus) was used for three sorts, and a total of nine sorts were used for the experiment, respectively. In addition, each tea extraction condensed by the evaporator

and was performed by [ as extracting for 10 minutes, adding 200ml of about 90-degree C boiling water to 10g of tea leaves, and adding churning suitably and freeze-drying extraction liquid after that ].

[0029] What took into the GLUT4 transformer location experiment what ****(ed) to KRH so that it might become the taking-in experiment of glucose in ml and 5mg /, and ****(ed) it to Phosphate buffered saline (PBS) by an experiment and this concentration again was used, respectively. As "catechin", on the other hand, catechin (C), EPIKATEKIN (EC), A total of eight sorts of GAROKATEKIN (GC), epigallocatechin (EGC), catechin gallate (Cg), epicatechin gallate (ECg), GAROKATEKIN gallate (GCg), and epigallocatechin gallate (EGCg) were used. These took in what was dissolved in dimethyl sulfoxide (DMSO) so that it might be set to 10mM, and used it for the experiment and the GLUT4 transformer location experiment.

[0030] (Cultivation of precursor adipose cell stock 3T3-L1 cell, and specialization guidance to adipose cells) After culturing 3T3-L1 cell which is a precursor adipose cell stock of mouse origin until it carried out sowing to 35mm or a 100mm dish and was saturated, specialization guidance to adipose cells was performed. Specialization guidance is 1microM. dexamethazone, 0.5mM 3-isobuthyl-1-methylxantine, The cell was cultured for three days by the DMEM culture medium (fetal calf serum (FBS) is included 10%) which added a 10microg [/ml ] insulin and 100microM ascorbic acid phosphorus acid, and it cultivated for two days by DMEM which subsequently contains a 10microg [/ml ] insulin and 100microM ascorbic acid phosphorus acid. Then, it was used for the experiment of ******** in the stage to which cultivation was continued for five to eight days by the DMEM culture medium which contains FBS 10%, and about 90% of cell specialized in adipose cells by microscope observation.

[0031] (Taking in into the cell of 3-OMG) After carrying out sowing of the 3T3-L1 cell to the 35mm dish and making it specialize in it, ****** was performed by cultivating by a non-serum culture medium beforehand for 18 hours. The influence of a tea extract and catechin was investigated using two kinds of methods shown below. Before the 1st method gives an insulin, it adds into a cell and a tea extract (100microg/ml ******) or catechin (50microM) For 15 minutes, Subsequently, after carrying out the object for catch cropping of the 100nM insulin for 15 minutes, 3-OMG (6.5mM, 0.5microcurie) which carried out the label by 3H was made to take in for 30 seconds. After another method added the 100nM insulin for 15 minutes into the cell beforehand and carried out the transformer location of GLUT4, it added the tea extract and took in 3H-3-OMG. Any method carried out taking in of 3H-3-OMG to a cell for 30 seconds, and stopped the reaction by washing a cell quickly with the KRH solution of 0.3mM FURORECHIN which is inhibitor of GLUT. Furthermore, the KRH solution of 0.3mM FURORECHIN washed the cell 3 times, and after solubilizing in SDS solution 0.5%, the value of 3H taken in in the cell by liquid scintillation counters was measured. As ****, adsorption into the nonspecific cell of 3H-3-OMG was beforehand measured similarly using the cell which checked taking in by FURORECHIN. What pulled the **** value from the value of 3H taken in in the cell was made into the true amount of glucose taking in.

[0032] (Manufacture of a cell membrane, and detection of GLUT4 by western blotting) [ detection of GLUT4 by western blotting ] It ****** like the above to 3T3-L1 adipose cells which carried out sowing to the 100mm dish, and were made to specialize in it. Tea extract processing for 15 minutes ([ 200ml of about 90-degree C boiling water / add and ]

to 10g of the above-mentioned tea extracts, i.e., tea leaves) The tea extract which extracted for 10 minutes, adding churning suitably, condensed extraction liquid by the evaporator, was made to freeze-dry it after that, and was obtained was made to act 50microg/ml. It carried out and what subsequently performed the insulin stimulus for 15 minutes was used. These cells were homogenized and a part for cell membrane drawing was prepared by the density gradient ultra-centrifugal separation method. The amount of protein for the obtained cell membrane drawing was measured, SDS-PAGE was presented with the 1microg, and protein was separated. The protein after separation is transferred on a PVDF (poly BINIRIDENJI fluoride) film. It blocked with the Tris-buffered saline-Tween (TBST:20mM Tris-HCl (pH 8.0), 0.15M NaCl, and 0.05% Tween20) solution of skim milk 5%. After washing a membrane several times by TBST, the anti-goat IgG antibody which carried out the hose radish par oxidase sign of the anti-GLUT4 antibody as a secondary antibody was made to react as a primary antibody. It is ECL about the immunity complex on a membrane. It was made to react with a plus chemistry luminescence reagent, and GLUT4 were detected by making an X ray film exposed.

[0033] (Detection of phosphorylation of the insulin receptor by an immunity precipitation method) [ the influence of the tea extract to phosphorylation of an insulin receptor (IR) ] the processing (tea extract processing for 15 minutes (the above-mentioned tea extract -- )) same to 3T3-L1 adipose cells which carried out sowing to the 100mm dish, and were made to specialize in it as the above Namely, it extracts for 10 minutes, adding 200ml of about 90-degree C boiling water to 10g of tea leaves, and adding churning suitably. The tea extract which condensed by the evaporator, was freeze-dried after that, and obtained extraction liquid was made to act 50microg/ml. It carried out and what subsequently performed the insulin stimulus for 15 minutes was used. These cells were dissolved by RIPA buffer (50 mM Tris-HCl (pH 8.0), 150mM NaCl, and 1%NP40, 0.5% deoxycholate, 0.1%SDS), and all the extraction liquid was prepared. The obtained extraction liquid measured the amount of proteins, carried out immunity sedimentation of the protein for 1mg by the anti-IR-beta antibody, and was made to adsorb the IR-beta subunit combined with the antibody to protein A/G agarose. The IR-beta subunits combined with the antibody according to centrifugal separation were collected, SDS-PAGE was presented and the western blotting using the anti-phospho tyrosine antibody as a primary antibody was presented after separating protein. The procedure of western blotting was performed like the above and used the anti-rabbit IgG which carried out the hose radish par oxidase sign as a secondary antibody.

[0034] In order to investigate the influence of the tea extract in a <result and consideration> (it is about ****** influence to Latt body fat of green tea) animal individual level, The following results were obtained, when Latt was made to do free ingestion of the green tea for three weeks and change of the ingredient in blood and change of the taking-in activity of glucose in adipose tissue and the muscular system were investigated.

[0035]

[Table 1]

| | コントロール | 緑　茶 |
|---|---|---|
| 体重(g)最終 | 146.8 ±6.3 | 144.4±13.1 |
| 摂食(g)平均 | 17.0 ±4.6 | 16.0± 4.3 |
| 摂水(茶)(ml)平均 | 21.2 ±6.0 | 22.2± 6.8 |
| 組　織 | 比体重(%) | |
| 脂　肪 | 2.68±0.06 | 2.35±0.11* |
| 肝　臓 | 7.96±0.27 | 7.42±0.68 |
| 脾　臓 | 0.54±0.03 | 0.53±0.04 |
| 胸　腺 | 0.41±0.02 | 0.39±0.09 |
| 腎　臓 | 1.42±0.33 | 1.34±0.09 |

Valules are means±S.D.(n=5)
* Significant difference from control group.
(p<0.05, by Student's t-test)

[0036]
[Table 2]

| | コントロール | 緑　茶 |
|---|---|---|
| トリアシルグリセロール(mg／dl) | 115.2±4.3 | 100.8±8.2 |
| NEFA(meq／dl) | 2.9±0.3 | 1.9±0.2* |
| 総コレステロール(mg／dl) | 105.0±1.0 | 84.0±2.0* |
| HDLコレステロール(mg／dl) | 51.5±1.6 | 42.0±1.9* |
| LDLコレステロール(mg／dl) | 20.5±1.2 | 14.5±1.2* |
| レプチン(ng／ml) | 8.8±0.5 | 7.7±0.5 |
| グルコース(mg／dl) | 196.0±4.5 | 204.0±7.6 |

Valules are means±S.D.(n=5)
* Significant difference from control group.
(p<0.05, by Student's t-test)

[0037]
[Table 3]

| | $^3$H-3-OMG取り込み(nmol/組織100mg) | |
|---|---|---|
| | コントロール | 緑　茶 |
| 脂肪 | 16.2±2.6 | 13.9±1.7 |
| 筋肉 | 22.8±1.5 | 27.6±1.4* |

Valules are means±S.D.(n=5)
* Significant difference from control group.
(p<0.05, by Student's t-test)

[0038] (Change of the Latt adipose tissue weight by tea ingestion) Comparison of weight change with the control group which carried out free ingestion only of the Latt group who made the tea extract take in, and the ion exchange water did not accept the significant difference in any during a breeding period and in front of slaughter ( drawing 1 , Table 1). Moreover, similarly change of the amount of food ingested between these 2 groups and the amount of drinking water was not accepted ( drawing 2 , Table 1). After slaughtering these Latt, when organization weight was measured, although the significant difference was not accepted, the weight of adipose tissue decreased about 15%. On the other hand, change was not accepted in the weight of liver, a spleen, and the thymus and the kidney. Moreover, in the observation in the naked eye, abnormalities were accepted in no internal organs including these. From these results, Latt's weight did not change by ingestion of the tea extract, but it was judged that the effect of becoming thin to the

animal of a normal growth phase was not accepted. However, the weight of adipose tissue showed the downward tendency. This suggests a possibility that a tea extract will control the specialization guidance to the adipose cells of a precursor adipose cell, and a possibility of decreasing the amount of adiposity of the adipose cells after specialization.
[0039] (Change of the plasma ingredient by tea ingestion) The plasma ingredient separated from above-mentioned Latt was measured. Change of the blood sugar level by ingestion of a tea extract was not accepted. On the other hand, the amount of bird acyl glycerol at the time of tea extract ingestion decreased about 15% compared with the control group (Table 2). On the other hand, each of amounts of total cholesterol, amounts of HDL cholesterol, and amounts of LDL cholesterol fell [ the amount of free fatty acid ] intentionally about 20% 35%. These results suggest that the ingredient of a tea extract has affected lipid metabolism in the living body. At least, since the main internal organs and the organization of bird acyl glycerol or cholesterol metabolism are liver, adipose tissue, and a small intestine, it is clear that the tea extract is performing absorption of a lipid ingredient or regulation of a composition / decomposition system in these internal organs and organizations.
[0040] As a result of investigating change of 3H-3-OMG taking in by adipose tissue and the muscular system, (Influence of the tea exerted on glucose taking in in adipose tissue and the muscular system) [ tea ingestion group Latt ] It is in the tendency to decrease taking in of 3H-3-OMG in adipose tissue, and the taking in was made to increase intentionally conversely in the muscular system (Table 3). It is thought that reduction of taking in of glucose in the adipose tissue of Latt of a tea ingestion group is closely connected with the downward tendency (Table 1) of lipid weight. Moreover, since the rise of the blood sugar level was not accepted in Table 2, it is thought that the glucose which was not taken into adipose tissue is actively taken into the organization of muscles or others.
[0041] (It is the influence of a ******** extract to glucose transportation of 3T3-L1 adipose cells) Adipose tissue weight decreasing by ingestion of a tea extract, and affecting lipid metabolism from the above-mentioned result, in the living body was shown. Adipose tissue is a place which bears raw composition of lipid from sugar. Then, the following results were obtained when it investigated what kind of influence the sugar transport mechanism of adipose tissue would receive by a tea ingredient on a cell level using the 3T3-L1 adipose-cell stock.
[0042] (It is ****** influence to glucose taking in of nine sorts of tea extracts) 3-OMG taking in of adipose cells increased by about 3 times by insulin stimulus, and has checked that the glucose transport mechanism of an insulin response existed in this cell. When a tea extract (100microg/(ml)) was made to act 15 minutes ago rather than an insulin, nine sorts of all tea extracts used for this experiment reduced the taking-in activity of 3-OMG under insulin existence ( drawing 3 ). When taking in of 3-OMG under insulin nonexistence was measured collectively, oolong tea showed the tendency to promote. The control effect under insulin existence investigated the concentration dependability of the control effect among the expensive tea extracts paying attention to green tea ( drawing 4 ). When a green tea extract was added before an insulin, taking in was decreased in concentration dependence and the effect was accepted also by the low concentration of 2microg/ml. Next, the influence of 3---OMG-taking in and passing through of the green tea extract was investigated in the cell which gave the insulin

stimulus beforehand. A green tea extract (100microg/(ml)) will decrease taking in promptly and temporally by operation time 1 minute ( drawing 5 left). Moreover, when the concentration dependability for operation time 1 minute was investigated, taking in was decreased more than in 5microg/ml ( drawing 5 right). Even if it added after giving, even if it added the tea extract from the above thing before it gave the insulin stimulus, it became clear that there is an effect which controls taking in of 3-OMG.
[0043] (It is ****** influence to the GLUT4 transformer location of a tea extract) [ adipose cells ] Since GLUT4 carried out the transformer location (shift) and the amount of taking in of glucose increased by stimulus of the insulin, it was expected that a tea extract acts on the communication-of-information course of this transformer location. Then, the influence of a ******** extract was investigated to the influence of a tea extract on the transformer location of GLUT4, and phosphorylation of the IR-beta subunit which is the Mogami style portion. When the transformer location of GLUT4 was investigated, the insulin increased remarkably the amount of GLUT4 protein on a cell membrane. When three sorts of green tea extracts were made to act before an insulin stimulus, the transformer location to the cell membrane of GLUT4 by an insulin was controlled ( drawing 6 ). The control effect of green tea and highest-quality green tea was stronger than that of coarse tea. Moreover, with tea, NUARA showed the control effect that a color type and a narcissus were the same in oolong tea, and, as for most influences on a transformer location, Dimbula of tea, Uva, and the iron Kannon of oolong tea were not seen. Next, as for the cell on which green tea was made to act, phosphorylation of beta subunit was looked at by the insulin like [ when the influence of phosphorylation on the IR-beta subunit of a green tea extract is investigated with an immunity precipitation method ] the control cell ( drawing 7 ). Furthermore, in the cell on which green tea was made to act, the quantity of the IR-beta subunit itself is the same as a control cell, and change was not accepted. Furthermore, when phosphorylation of the tyrosine residue was investigated using all the protein of a cell, the band near [ equivalent to the molecular weight of an IR-beta subunit ] 95kDa received phosphorylation by the insulin. However, the difference in the phosphorylation grade by a green tea extract was not accepted. The above result showed that a green tea extract did not change phosphorylation of IR. Therefore, adjusting glucose transportation was suggested, without it showing the initial signal to an insulin that a tea extract does not affect combination of RESEPUTAHE of an insulin, but controls the transformer location of GLUT4 specifically, i.e., a tea extract.
[0044] (It is ****** influence to glucose taking in of catechin) [ ****** influence ] as compared with taking in of the glucose which a tea extract shows, and the transformer location of GLUT4 Since the action changed with brown kinds, it was possible that composition of ingredients, such as catechin contained in each extract, and the difference in content participate in action change of a glucose transport mechanism. Then, the following result was able to be obtained when investigated about the influence eight sorts of catechin has on 3H-3-OMG taking in. Four sorts of catechin (Cg, ECg, GCg, EGCg) which has gallate ester among catechin decreased the amount of glucose taking in under insulin existence, and other four sorts (C, EC, GC, EGC) did not decrease it ( drawing 8 ). The effect of Cg was the strongest in four sorts in which control was accepted. It was suggested that catechin gallate (Cg) acts on the glucose transport mechanism of 3T3-L1 adipose cells from these things also especially in catechin in a tea extract, especially catechin with gallate ester. Moreover, although the theaflavin which is a polymerization

thing was contained in tea in Toyotomi, when taking in of 3-OMG was investigated about the theaflavin mixture, the quite strong control effect was accepted.
[0045] When Latt was made to take in a <conclusion> tea extract, although weight did not change, adipose tissue weight showed the downward tendency. Adipose tissue is the place of raw composition of sugar to lipid, and if sugar transportation activity falls or fat solution activities, such as nature lipase of lipoprotein and hormone susceptibility lipase, increase, it will lead to weight reduction of adipose tissue. Since accumulation of fat participates in the onset of lifestyle-related diseases, such as diabetes, the ingestion of a tea extract can expect the preventive effect of a lifestyle-related disease. Moreover, since normal Latt immediately after ablactation (3 marriageable age) was made to take in tea, the downward tendency of adipose tissue weight has a high possibility of being based on the differential inhibition of the adipose cells through control of sugar transportation activity. It was possible that a tea extract controls the sugar transportation to adipose tissue, and prevents supply of excess energy after the blood sugar level increased in connection with ingestion and the insulin from a pancreas beta cell has increased, since the tea extract controlled the glucose transportation activity under insulin existence by the cultured cell system. On the other hand, although the bad influence of becoming hyperglycemia was expected in connection with this control effect, by tea ingestion Latt, change was not accepted in the blood sugar level, but taking in of glucose of the muscular system was accelerating. It is possible that tea turns excess energy to a motile organ, and is acting on improvement in activity from this. although muscles and adipose tissue have specialized from the same ****** and this discovers both GLUT4, it is ****** about the opposite action being shown with a tea extract in the organ and organization in which the isoforms of the same GLUT differ. Moreover, it accepted as the catechin which has the inside of a tea extract or catechin, especially gallate ester also for the above effect as a ****** active ingredient, and the thing which demonstrates the effect that catechin gallate is strong, also especially in it.
[0046] Glucose taking-in inhibitor [ in / by the following prescription / adipose cells ], (Work example 1) The tablet as insulin stimulus response nature glucose taking-in inhibitor, the GLUT4 transformer location depressant in adipose cells, the glucose taking-in activating agent in muscle cells, the GLUT4 transformer location activating agent in muscle cells, or a muscular activating agent was created.
[0047]
Tea extract (Thea Fran 90S or Thea Fran 30A) -- 120mg vitamin C -- 50mg emulsification oligosaccharide -- 90mg granulation agent -- 60mg microcrystalline cellulose -- 80mg reduction maltose starch syrup -- 90mg sucrose -- 100mg spice -- Proper-quantity [0048] = (Work example 2) By the following prescription, the drink as fat mitigation food and drink or muscular activity food and drink was created.
[0049]
Catechin gallate -- 50mg vitamin C -- 50mg fructose grape liquid sugar -- 10g water solubility dietary fiber -- 500mg spice -- Proper-quantity ion exchange water -- 100mL

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[Translation done.]